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TITLE: A Study of Transrectal Tumor Oxygen Measurements in Patients Which Clinically Localized Prostate Cancer

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13. ABSTRACT (Maximum 200 Words)

The primary aim of this clinical study is to determine the relationship between pre-treatment prostate cancer oxygen levels and long-term disease control following treatment with radiotherapy, and the independent prognostic effect of oxygen measurements relative to established prognostic factors. In addition, the study will determine the relationship between pre-treatment tumor oxygen levels and mutations of the p53 tumor suppressor gene, and the impact of this interaction on patient outcome. It is anticipated that the accrual target of 195 patients will be achieved by July 31, 2005. The oxygen measurement technique was revised based on our pilot study to assure that the highest quality data are being collected. The microregional distribution of oxygen in prostate cancer biopsies will be studied using intrinsic markers of oxygenation. The molecular studies of p53 are proceeding as outlined in the proposal. A preliminary analysis suggested a potentially important influence of prostrate cancer hypoxia on patient outcome following treatment with radiotherapy, in agreement with the underlying hypothesis of the study. However, longer patient follow-up is required to confirm this early result. It is anticipated that all aspects of the proposed work will be completed.

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Introduction

Hypoxia is known to impair the effectiveness of both surgery and radiotherapy at curing a variety of solid human tumors. This may result from an hypoxia-induced increase in genetic instability leading to altered expression of genes that are important in tumor growth and progression. These genetic changes are manifested clinically as more aggressive tumor behavior. The primary aim of this clinical study is to determine the relationship between pre-treatment prostate cancer oxygen levels and long-term disease control following treatment with radiotherapy, and the independent prognostic effect of oxygen measurements relative to established prognostic factors. In addition, the study will determine the relationship between pre-treatment tumor oxygen levels and mutations of the p53 tumor suppressor gene, and the impact of this interaction on patient outcome.

Body

Progress in the third year of the research award has focused on Task 1 of the Statement of Work:

Task 1. Accrual of patients (years 1-3)

Patients will be accrued to this study at a uniform rate of 65 per year (52 eligible patients per year allowing for 20% attrition), over the three years from January 2001 to December 2003. Clinical and surgico-pathologic prognostic information will be collected prospectively at the time each patient enters the study. Eppendorf oxygen measurements will be made. A biopsy will be obtained immediately after the oxygen measurements and evaluated for mutations of the p53 tumor suppressor gene and apoptosis. The biopsies will be processed in batches during the accrual period. A portion of each biopsy will be stored for future study of other hypoxia-related genes.

Patients will be accrued to the EF-5 component of the study in the second and third years once phase I testing of this agent is complete in Canada.

Accrual to this clinical study began in August 2001 after the protocol was approved by the Human Subjects Research Review Board of the U.S. Army Medical Research and Materiel Command, and our local institutional Research Ethics Board. The projected accrual was 65 patients annually, to achieve a total study population of 195 patients (156 eligible patients after allowing for attrition). The study has now been open for three years.

Accrual to the study was slower than expected in 2002 and 2003 because of the Severe Acute Respiratory Syndrome (SARS) epidemic in Toronto that incapacitated all but emergency health care delivery. All clinical (and a large proportion of laboratory research) was completely shut down for several weeks, and significantly curtailed for an even longer period, in an attempt to control the spread of the infection. This prevented us from accruing patients to this clinical study of men with prostate cancer. In addition, there was a delayed effect of the epidemic: a backlog of patients with suspected prostate cancer developed because of limited access to biopsy facilities during the period of the outbreak, and the number of patients presenting for curative treatment with radiotherapy was correspondingly reduced. A request was therefore submitted in November 2003 to extend the period of accrual to the study by one year, with the revised accrual period to end July 31, 2005 (see letter to Ms. Sherri Walters dated November 23, 2003). No additional funds were requested. This extension was approved by the U.S. Army Medical Research and Materiel Command on December 9, 2003.

As of July 31, 2003, a total of 149 patients had been accrued to the study:

Year 1	40 patients
Year 2	65 patients
Year 3	44 patients

Therefore, a further 46 patients will be required in year 4 to meet the accrual target of 195 patients. We anticipate no difficulty in achieving this by July 31, 2005.

One patient accrued to the study in 2004 had a Serious Adverse Event. He developed uro-sepsis on June 23, 2004 after trans-rectal ultrasound of his prostate gland, oxygen measurements (as per the study protocol), prostate biopsies (as per the study protocol), and radio-opaque marker seed insertion (routine clinical care). He received intra-venous antibiotics. He subsequently had a myocardial infarction that necessitated hospitalization. This was uncomplicated and he recovered without further problems. He is well at present. The relationship of this event to the study interventions (prostate oxygen measurements and biopsies) is uncertain. All patients who receive radiotherapy for prostate cancer at this institution have radio-opaque marker seeds inserted trans-rectally under ultrasound guidance regardless of whether or not they consent to participate in this study. Therefore, he would have been exposed to the same risk of infection had he chosen not to participate. All precautions were followed in this case to minimize the risk of infection (eg. the use of prophylactic antibiotics in advance of the procedure as outlined in section 4.4 of the study protocol). This is the only serious adverse event in 149 patients accrued to the study. It was reported to the University Health Network (UHN) Data Safety Monitoring Board, and the UHN Research Ethics Board. It was reported to the U.S. Army Medical Research and Materiel Command on July 5, 2004. No change to either the research protocol or consent form was deemed necessary.

The research protocol was revised in 2003-4 to reflect new results from an independent pilot study conducted at this institution. The details of the revision are outlined in the previously submitted amended protocol dated November 21, 2003. The revised protocol specifies 4 oxygen measurement tracks. The biopsies from along the measurement tracks were eliminated. A single biopsy will still be obtained from the region of the measurements for p53 DNA sequencing and immunohistochemical studies. It is anticipated that these changes will improve the quality of the data without impacting on the experience of patients participating in the study. The total number of needle punctures will remain unchanged at 5. The duration of the procedure will remain unchanged. There should be no difference in the very low risk of bleeding or infection. This amendment was approved by the U.S. Army Medical Research and Materiel Command on January 12, 2004.

A minor component of the project involves the use of the hypoxia marker EF5. As outlined in Section 4.6 of the protocol, this was to be administered to 30 patients in years 2 and 3 to evaluate the microscopic distribution of oxygen in prostate cancer, and differences in gene expression between oxic and hypoxic regions. This component of the proposed work has been revised to reflect significant development in the area of intrinsic hypoxia markers. These are normal proteins that are known to be up-regulated in the setting of hypoxia, and can be used in the same manner as EF5 to evaluate micro-regional aspects of tumor oxygenation. The intrinsic markers have several advantages, in that administration of an external agent prior to biopsy is not required and the immunohistochemical analysis can be done on previously-obtained paraffin embedded tissue. We will use the intrinsic markers carbonic anhydrase IX (CA-IX), glucose transporter (GLUT-1) and hypoxia inducible factor-1α (HIF-1α) to accomplish the goals of this project (1-5). The analysis technique for the instrinsic markers will not differ significantly from that originally proposed for EF5, and we therefore do not anticipate any change to the budget. These changes were included in the revised protocol of November 21, 2003. The amended protocol was approved by the U.S. Army Medical Research and Materiel Command on January 12, 2004.

The component of the project investigating the relationship between prostate cancer oxygenation, p53 status and downstream activation of p53 related pathways (section 4.5 of the protocol) are progressing as planned. The laser-capture micro-dissection, DNA sequencing and immunohistochemistry techniques have been developed, tested and refined. The patient samples will be processed as a batch once accrual to the study is complete to assure a consistent analysis approach.

Task 2. Follow-up (years 4-7)

Patients will be followed for a duration of 3.5 years after completion of accrual in order to realize the required 46 PSA relapses. Patients will be assessed clinically and have PSA measured at regular intervals as part of their routine medical care. The database will be updated on an ongoing basis to reflect current disease and patient status.

Patients who were accrued to the study in years 1 to 3 are being followed after the oxygen measurements and radiotherapy as outlined in section 4.8 of the protocol. Regular review of the patient data is performed to assure completeness and accuracy.

Task 3. Analysis (years 4 and 7)

The comparison of tumor oxygenation to other clinical and surgico-pathologic prognostic factors will be done after completion of accrual (early in year 4). The analysis of the influence of tumor hypoxia on outcome will be done after patients have been followed for an additional 3.5 years (mid year 7).

A preliminary analysis was done in 2003-4. The effect of prostate cancer oxygen levels on the outcome of 150 patients accrued to either the previous pilot study or the current study was evaluated. All patients were treated using escalated dose conformal radiotherapy, with or without neoadjuvant and concurrent hormonal therapy. Patients with hypoxic tumors had a lower PSA-failure free rate than those with better oxygenated tumors. This supports the hypothesis that hypoxia is an important adverse biologic prognostic factor in prostate cancer. However, the follow-up of patients accrued to this study remains short at less than 2 years, and further time is required to reliably determine the influence of tumor oxygenation on patient outcome.

The results of this preliminary analysis were presented at the 2004 annual meetings of the American Society for Therapeutic Radiology and Oncology (Appendix 1) and the Canadian Association of Radiation Oncologists (Appendix 2).

Key Research Accomplishments

1. Ongoing patient accrual in alignment with that predicted in the original study proposal. It is anticipated that the accrual target of 195 patients will be achieved by July 31, 2005.
2. Follow-up of patients accrued to the study in years 1 to 3.
3. Revision of the oxygen measurement technique based on the results of our pilot study, to assure that the highest quality data are obtained.
4. Revision of the technique for studying the microscopic distribution of hypoxia in prostate cancer based on evolving knowledge of intrinsic tissue markers of hypoxia.
5. Preliminary analysis demonstrating a potentially important influence of prostate cancer oxygenation on patient outcome following radiotherapy, in agreement with the hypothesis of the study.

Reportable Outcomes

Preliminary analysis demonstrating a potentially important influence of prostate cancer oxygenation on patient outcome following radiotherapy, in agreement with the hypothesis of the study.

Conclusions

This study of oxygenation in human prostate cancer continues to accrue patients. It is anticipated that the accrual target of 195 patients will be achieved by July 31, 2005. The oxygen measurement technique has been revised based on our pilot study to assure that the highest quality data are being collected. The microregional distribution of oxygen in prostate cancer biopsies will be studied using intrinsic markers of oxygenation rather than EF5 as described in the initial proposal. The molecular studies of p53 are proceeding as outlined in the proposal. A preliminary analysis has suggested a potentially important influence of prostrate cancer hypoxia on patient outcome following treatment with radiotherapy, in agreement with the underlying hypothesis of the study. However, longer patient follow-up is required to confirm this early result.

Overall, the aims of this study are progressing as planned and it is anticipated that all aspects of the project will be completed.

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Appendix 1

2004 Annual Meeting of the American Society for Therapeutic Radiology and Oncology (ASTRO)

Milosevic M, Bristow R, Chung P, Panzarella T, Toi A, Hill R. Prostate cancer hypoxia correlates with poor patient outcome following treatment with radiotherapy (Abstract). Int J Radiat Oncol Biol Phys 60(Supp), S236, 2004.

Prostate Cancer Hypoxia Correlates with Poor Patient Outcome Following Treatment with Radiotherapy

M. Milosevic, R. Bristow, P. Chung, T. Panzarella, A. Toi, R. Hill, for the Radiation Medicine Program Genitourinary Oncology Group, Princess Margaret Hospital, Toronto, Canada

Purpose: Tumor hypoxia is an important biologic determinant of patient outcome in a number of human malignancies. It has been associated with genetic instability, malignant progression and the development of metastases. The aim of this study was to evaluate the influence of tumor hypoxia on the outcome of patients with prostate cancer treated in a uniform way with high-dose conformal radiotherapy.

Methods: A total of 143 patients with clinically localized (cN0, cM0) prostate cancer underwent transrectal, ultrasound-guided measurement of tumor oxygen levels using the *Eppendorf* polarographic electrode system. Between 40 and 80 individual oxygen measurements were obtained from tumor-bearing regions of the prostate gland in each patient prior to treatment. Hypoxia was expressed as the percentage of oxygen values <5 mm Hg (HP_5). Most of the patients had cT1-2 tumors (97%), a Gleason score of 6 or 7 (90%), and a PSA <20 (89%). Neoadjuvant hormonal therapy was administered to 42 (29%) for 1-3 months prior to the oxygen measurements. All patients were treated using conformal radiotherapy to a dose of either 75.6 or 79.8 Gy in 42 daily fractions. The median follow-up was 1.8 years.

Results: The HP_5 in individual patients ranged from 0-100%, with a median of 38%, similar to other human tumors. There was no correlation between HP_5 and T-category, Gleason score, pre-treatment PSA or prior hormonal therapy. There were 17 PSA failures using the ASTRO consensus definition. High HP_5, when analyzed either as a continuous variable or dichotomized about the median value, was associated with a high risk of PSA progression (p=0.03 and 0.02 respectively). The 2-year actuarial PSA failure-free rate was 92% in patients with oxic tumors ($HP_5 \leq 38\%$), versus 72% in those with hypoxic tumors ($HP_5 > 38\%$). Clinical prognostic factors, prior hormonal therapy and radiation dose were balanced between the oxic and hypoxic groups.

Conclusions: The early results of this prospective study support the hypothesis that tumor hypoxia is a clinically important biologic parameter that influences the outcome of patients with localized prostate cancer treated with external beam radiotherapy. This has potentially important implications for the development of future management strategies that specifically target hypoxia in this disease. Our study continue to accrue and mature, and will ultimately allow us to rigorously evaluate the independent prognostic important of hypoxia in these patients, the patterns of recurrence in patients with oxic and hypoxic tumors, and the influence of oxygen levels on the interaction between hormonal therapy and radiation.

Appendix 2

2004 Annual Meeting of the Canadian Association of Radiation Oncologists (CARO)

Milosevic M, Chung P, Bristow R, Toi A, Panzarella T, Hill R. Prostate cancer hypoxia adversely influences outcome following treatment with radiotherapy (Abstract). Radiother Oncology 72 (Supp 1), S8, 2004.

Prostate Cancer Hypoxia Adversely Influences Outcome Following Treatment with Radiotherapy

M. Milosevic, P. Chung, R. Bristow, A. Toi, T. Panzarella, R. Hill, on behalf of the Radiation Medicine Program Genitourinary Oncology Group, Princess Margaret Hospital, Toronto, Canada

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